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14. ABSTRACT

The key to our experimental approach to the creation of Vascular Materials is the development of material syntheses and methods of microfabrication that allow us to embed microfluidic structure directly within hydrogels. For the operation and characterization of systems (Artificial Leaves, Wound Dressings, and Tissue Scaffolds) based on Vascular Materials, we have developed experiments to control and monitor fluxes of heat and mass in an automated fashion. In parallel, we have developed a theoretical basis for the design and operation of this new class of active materials.
Highlights:1) Development of techniques to embed functional microfluidic structure within organic hydrogels. 2) Development of a complete theory and experimental characterization of heat and mass transfer in transpiration from hydrated materials. This work is a foundation our development of a flexible heat pipe. 3) Development of an Active Wound Dressing that allows for external management of the mass exchange and mechanical stimulation of the wound bed. 4) Development of the first microfluidic scaffold for three-dimensional cell culture and tissue engineering.

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FINAL REPORT

GRANT TITLE: Vascular Materials for Human Heat Management and Wound Healing

PRINCIPAL INVESTIGATOR: Abraham D. Stroock

INSTITUTION: Cornell University

GRANT NUMBER: N00014-04-1-0652

AWARD PERIOD: 6/31/2004-12/31/2005

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OBJECTIVE: **1)** Develop general design principles and fabrication methods for synthetic Vascular Materials. **2)** Develop Vascular Biomaterials for the active control of the bio-synthetic interface with specific applications in wound healing and tissue engineering. **3)** Develop an Artificial Leaf, and exploit this structure in a high performance system for heat management on the human body, a Vascular Heat Belt.

Our second goal with regards to controlling mass transfer with biological systems has broadened to include the development of microfluidic scaffolds for tissue engineering. This goal is complementary in two ways to our primary goal of developing active wound dressings: 1) parallel development of fabrication methods in biological materials that are relevant to the wound healing context, 2) development of a chemically programmable interface with living cells that may be relevant to regeneration of skin for burn victims.

APPROACH:

The key to our experimental approach to the creation of Vascular Materials is the development of material syntheses and methods of microfabrication that allow us to embed microfluidic structure directly within hydrogels. For the operation and characterization of systems (Artificial Leaves, Wound Dressings, and Tissue Scaffolds) based on Vascular Materials, we have developed experiments to control and monitor fluxes of heat and mass in an automated fashion. In parallel, we have developed a theoretical basis for the design and operation of this new class of active materials.

ACCOMPLISHMENTS (for the entire 1.5-year period):

Highlights:

1) Development of techniques to embed functional microfluidic structure within organic hydrogels.

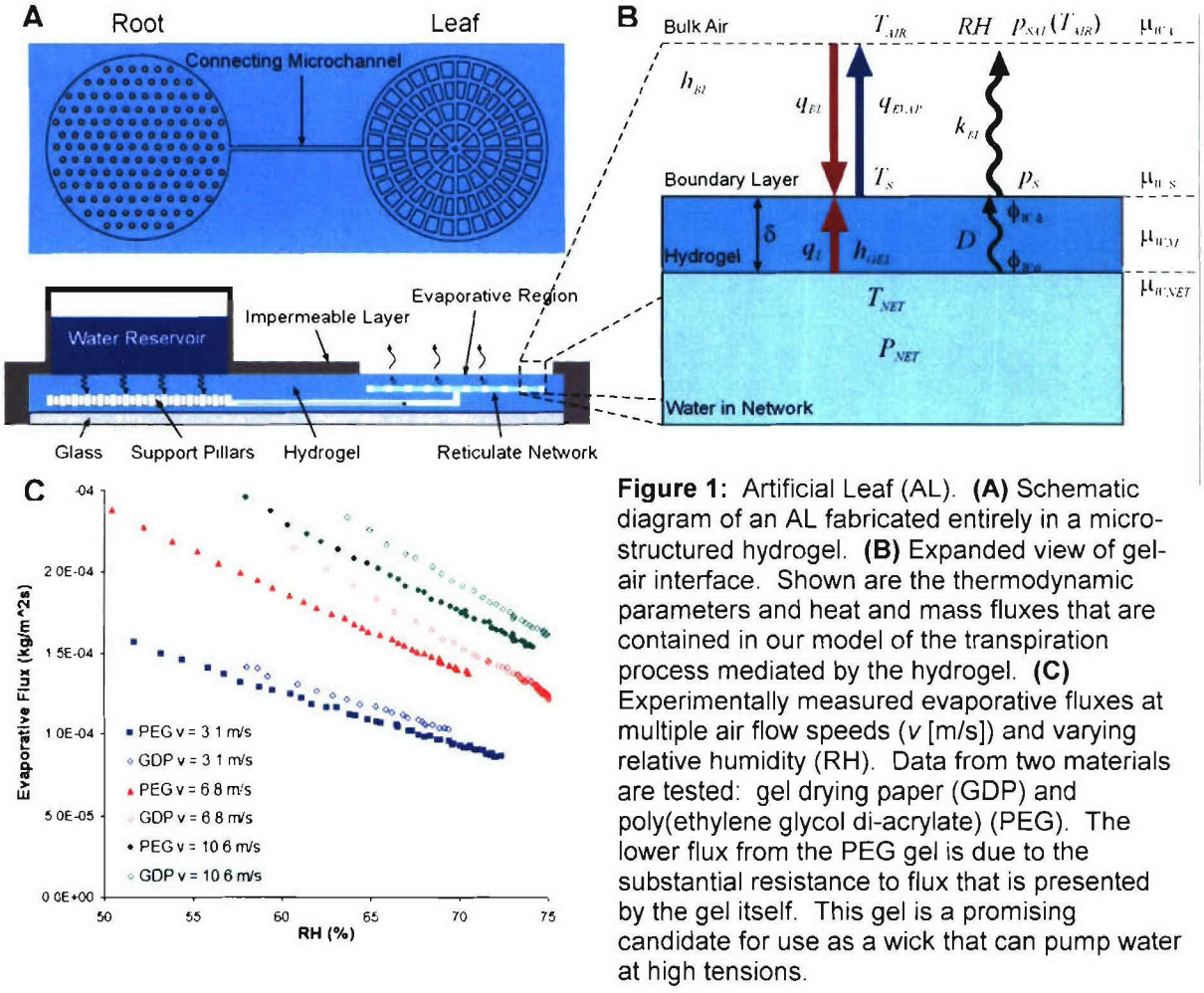

Figure 1: Artificial Leaf (AL). **(A)** Schematic diagram of an AL fabricated entirely in a micro-structured hydrogel. **(B)** Expanded view of gel-air interface. Shown are the thermodynamic parameters and heat and mass fluxes that are contained in our model of the transpiration process mediated by the hydrogel. **(C)** Experimentally measured evaporative fluxes at multiple air flow speeds (v [m/s]) and varying relative humidity (RH). Data from two materials are tested: gel drying paper (GDP) and poly(ethylene glycol di-acrylate) (PEG). The lower flux from the PEG gel is due to the substantial resistance to flux that is presented by the gel itself. This gel is a promising candidate for use as a wick that can pump water at high tensions.

2) Development of a complete theory and experimental characterization of heat and mass transfer in transpiration from hydrated materials. This work is a foundation our development of a flexible heat pipe.

3) Development of an Active Wound Dressing that allows for external management of the mass exchange and mechanical stimulation of the wound bed.

4) Development of the first microfluidic scaffold for three-dimensional cell culture and tissue engineering.

Expanded accomplishments:

We have made important progress on several fronts in the development of Vascular Materials for the management of the chemical and physical properties of the bio-synthetic interface.

In the development of Artificial Leaves (AL) to act as wicks in flexible heat pipes, we have completed the assembly and calibration of an environmentally controlled chamber in which to characterize the leaves, and we have begun to test hydrogels for use as the core material in the AL (Figure 1). Our method of characterizing the function of leaves depends on careful calibration of the convective transfer of heat and mass at the evaporative surface. Our current

experiments allow us to evaluate the chemical potential of the water throughout the system (Figure 1B) during steady state transpiration. With this measurement, we are able to know the mechanical state (i.e., the pressure or tension) in the liquid water in the leaf without requiring any direct mechanical connection. We are now in a position to be the first group to observe a synthetic wick move liquids under tension. This experimental system will also serve for the characterization of heat transfer in prototypes of heat pipes.

We have begun testing materials for use as wicks. Our initial tests have involved wicking water at ambient pressure with no hydraulic load. In these tests, the material itself acts as a substantial resistance to mass transfer, leading to the equivalent of many atmospheres of pressure drop across itself. The plots in Figure 1C compare the measured transpiration through a macroscopically porous wick (GDP) that presents negligible resistance to flow, and a molecular gel (PEG) that presents a massive resistance to flow. The despite the large resistance, the gel maintains fluxes that are within 20% of those of the paper. These results indicate that PEG gels are promising candidates for use as high performance wicks. We are pursuing tests of PEG gels with hydraulic loads in the geometry shown in Figure 1A.

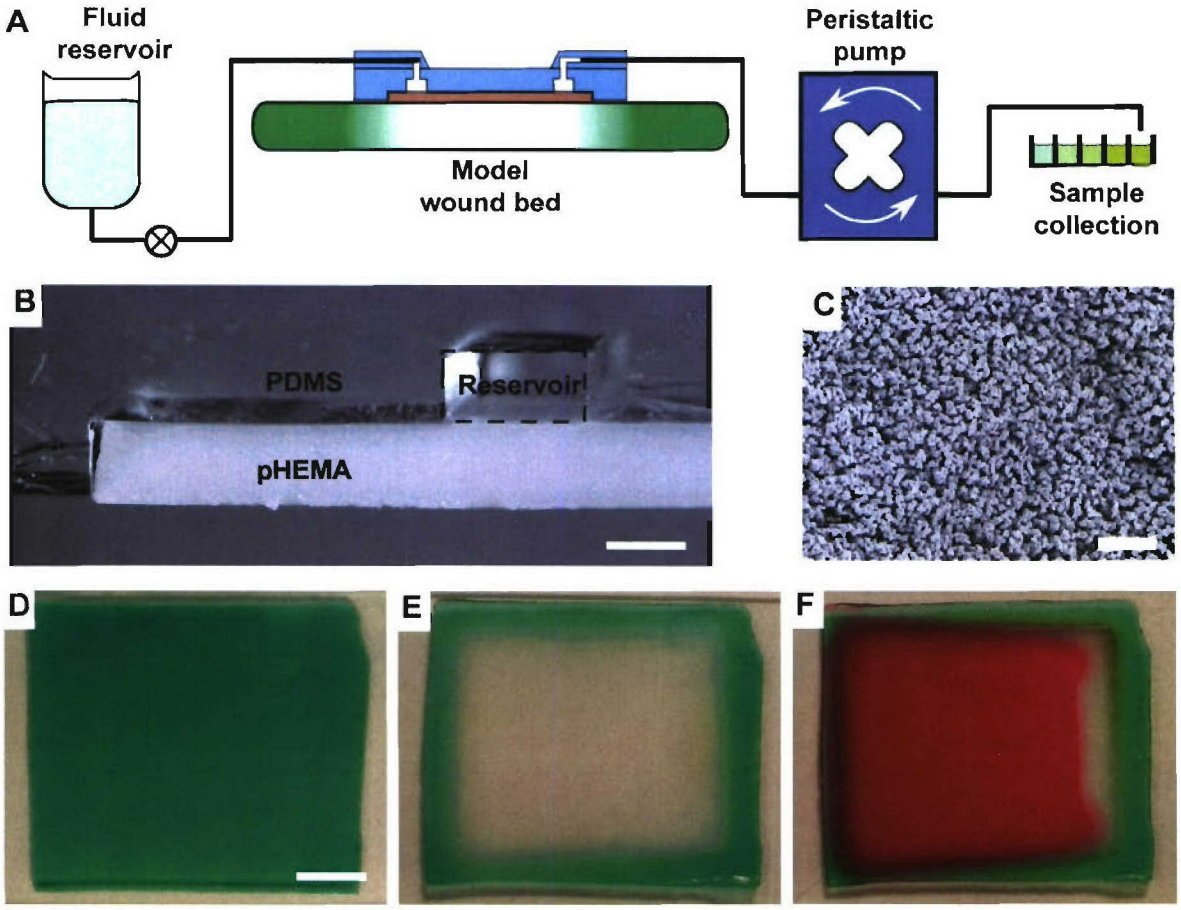

Figure 3: Active Wound Dressing (AWD). **(A)** Schematic diagram of operation of AWD on model wound bed. **(B)** Optical micrograph of cut cross-section of AWD showing bilayer structure of PDMS and porous poly(hydroxyethyle methacrylate) (pHEMA). **(C)** Scanning electron micrograph of porous pHEMA. **(D-F)** Illustration of the state of a model wound bed saturated with dye before (D), after extraction (E), and after subsequent delivery of a second dye (F).

In our work toward an Active Wound Dressing (AWD), we have made important steps in the development of materials, fabrication, and *in vitro* characterization. Figure 2A presents the mode of operation of this dressing. As originally proposed, our system is a hybrid of a hydrogel-based interface with the wound and a silicone backing. Figure 2B shows a cross-sectional cut of a AWD, showing the bilayer structure formed by a silicone (PDMS) backing and a porous hydrogel interface with the wound bed. We have focused substantial attention on the character of the hydrogel layer, such that we achieve well-controlled exchange of both molecular- and cellular-scale material with the wound bed. Figure 2C presents a scanning electron micrograph (SEM) of the surface of 1 mm-thick sheet of poly(hydroxyethyl methacrylate) (pHEMA) formed by phase separation polymerization. We have developed a thermally initiated process that leads to uniform porosity on the scale of 10-100 μm over areas of 100 cm^2. This porosity is important to allow for convection of insoluble materials from the wound bed; the uniformity of the interfacial is crucial to ensure uniform convective flux across the wound bed. This fabrication strategy is compatible with the addition of coherent microstructure via photopolymerization of neat pHEMA within the sponge in order to deliver spatially confined streams of fluid to the wound bed.

We have characterized of mass exchange mediated by AWDs. For this purpose, we have operated the AWD on model wound beds. One such system is a film of calcium alginate hydrogel that mimics both the modulus and the permeability of tissue. Figure 2D-F show an experiment run on this model wound bed. We initiate the experiment by infiltrating the model wound bed with a dye (Figure 2D). We then apply the AWD with a stream of clean solution to extract the dye from the substrate (Figure 2E). Finally, we perfuse the AWD with a solution of a second solute in order to deliver to the same substrate (Figure 2F). The extraction and delivery can also be run simultaneously. We have also demonstrated the ability to quantify the composition in the efflux from the AWD; this capability allows for diagnostic intervention without removing the dressing.

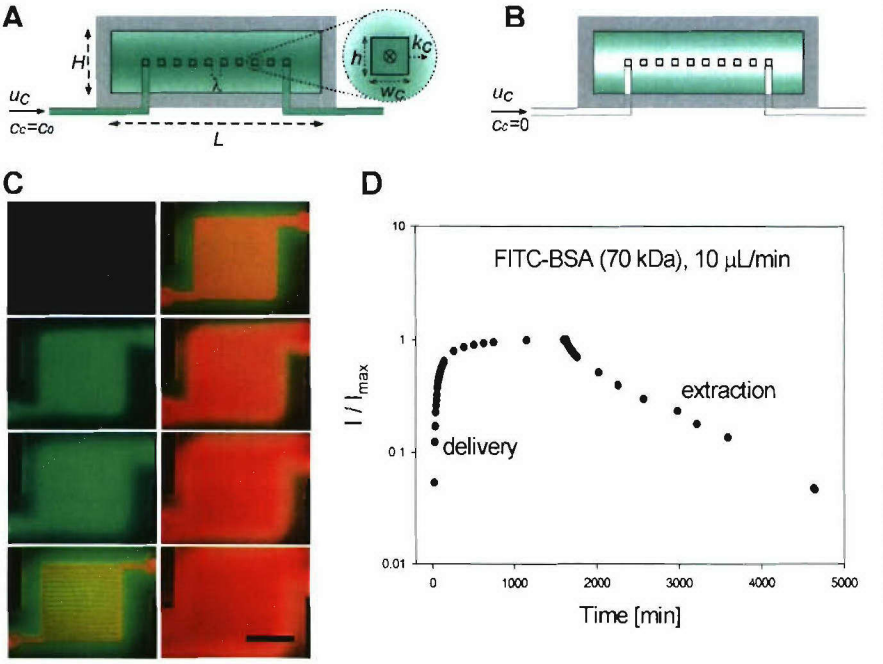

Figure 3: Microfluidic Scaffold in calcium alginate (4% w/v). **(A-B)** Cross-sectional views illustrating delivery (A) and extraction (B) of solute to and from gel via embedded microfluidic channels. **(C)** Fluorescent micrographs showing delivery of fluorescein followed by simultaneous delivery of rhodamine and extraction of fluorescein. Scale bar = 0.5 cm. **(D)** Transient concentration of fluorescently labeled bovine serum albumin during delivery and extraction as in (C).

Over the past year, we have initiated another exciting theme in the development of Vascular Materials: Microfluidic Scaffolds for 3-D cell culture and tissue engineering. This theme complements our work on AWDs as it addresses the longer term challenge of growing replacement tissues for victims of injuries such as burns. In this application, the microfluidic network provides convectively-aided exchange of mass within a cell-seeded volume defined by a biological material. To accomplish this goal, the microfluidic structure must be entirely embedded in a material that is convectively impermeable and highly diffusively permeable. We note that this is a distinct situation from that of the AWD for which the desirable material is convectively permeable, such that non-diffusive species can be exchanged. To the best of our knowledge, we are the first group to have created an appropriate structure.

We have achieved an appropriate microfluidic structure in calcium alginate gels of extremely low solid-fractions (4% w/v). The diagrams in Figures 3A-B illustrate modes of operation that we have used to characterized mass transfer in a-cellular Microfluidic Scaffolds. In these experiments, we use the microfluidic network to deliver (Figure 3A) and extract (Figure 3B) dyes from the three-dimensional volume defined by the gel. The fluorescence micrographs in Figure 3C show the power of this system for controlling the temporal evolution of the chemical state within the gel: In the first three frames on the left, fluorescein is delivered over a period of 1 hour; in the fourth frame, a stream containing rhodamine and no fluorescein is injected such that the dyes are exchanged simultaneously. Results reported previous illustrate the ability to substantially increase the rate of exchange of both small (fluorescein) and large (dextran) molecules with a material via an embedded microfluidic vascular structure (Cabodi et al., JACS 2005). We are currently adapting our methods to be sterile and generally cell compatible. Our efforts are focused on engineering the growth of cartilage *in vitro*. We are using primary bovine articular chondrocytes and equine mesenchymal stem cells within our microfluidic scaffolds. Our goal is to grow a monolithic tissue plug that contains the bone-cartilage interface.

Significance:

1) New techniques for embedding functional microstructure within soft, hydrated organic materials form a basis for an array of applications in thermal and chemical management.
2) Prototype of a convective wound dressing opens the way to a new active mode of wound management.
3) First microfluidic scaffold for 3D tissue culture allow unprecedented control of chemical environment experienced by cells in physiologically relevant architectures.

PUBLICATIONS, ABSTRACTS, TECHNICAL REPORTS, PATENTS, AND AWARDS (last 12 months):

Publications:

1) Stroock, A. D., Wheeler, T.D., and Kirtland, J.D. "Microfluidic relief for transport limitations." Biotechniques **39**(2): 159-163 (2005).
2) Cabodi, M.; Choi, N. W.; Gleghorn, J. P.; Lee, C. S.; Bonassar, L. J.; Stroock, A. D. A microfluidic biomaterial. *Journal of the American Chemical Society*, *127*, 13788-13789 (2005).
3) Stroock, A. D.; Cabodi, M. Microfluidic biomaterials. *MRS Bulletin*, **31**(2), 114-119 (2006).
4). Kenis, P. J. A. and Stroock, A.D.. "Materials for micro- and nanofluidics." MRS Bulletin **31**(2): 87-94 (2006).

Publications in revision or preparation:

1) Gleghorn, J. P.; Lee, C. S. D.; Cabodi, M.; Stroock, A. D.; Bonassar, L. J. Adhesive Properties of Laminated Alginate Gels for Tissue Engineering of Layer Structures. Journal of Biomedical Materials Research (in review).
2) Cabodi, M.; Cross, V. L.; Qu, Z.; Havenstrite, K. L.; Stroock, A. D. An active wound dressing for controlled convective mass transfer with the wound bed. (submission 7/2006).
3) Wheeler, T. D.; Stroock, A. D. Characterizing the transport of water across hydrogel membranes driven by evaporation.(in preparation).
4) Wheeler, T. D.; Stroock, A. D. Moving Liquid Water Under Tension with Synthetic Leaves (in preparation).
5) Choi, N. W.; Cabodi, M.; Bonassar, L. J.; Stroock, A. D. Microfluidic Scaffolds for Tissue Engineering. (in preparation).

Conference Abstracts:

1) Cabodi, M.; Choi, N. W.; Gleghorn, J. P.; Lee, C. S. J.; Bonassar, L. J.; Stroock, A. D. A Microfluidic Scaffold for Tissue Engineering. AICHE. Cincinatti. (2005)
2) Kirtland, J.; McGraw, G. J.; Stroock, A. D. Mass Transport to Boundaries and Mixing in Microfluidic Systems. AICHE. Cincinnati. (2005)
3) Wheeler, T. D.; Stroock, A. D. Pervaporation of Water through Poly(ethylene glycol) Hydrogels: The Pumping Mechanism of a Synthetic Leaf. AICHE. Cincinnati. (2005)
4) Cabodi, M.; Havenstrite, K.; Schwartz, S.; Stroock, A. D. A Microfluidic Wound Dressing and Wound Analysis Tool. ASME Summer Bioengineering. Vail. (2005)
5) Stroock, A. D.; Cabodi, M.; Lee, C. S. J.; Choi, N. W.; Bonassar, L. J. Tools and Concepts for Controlling Transport for In Vitro Engineering of Cartilage. ASME Summer Bioengineering. Vail. (2005)
6) Stroock, A.D. "Microfluidic Biomaterials," at 4th Air Force Workshop on Multifunctional Aerospace Materials and Structures, University of Illinois Urbana Champaign. (2005).

Patents:

1) Patent: Stroock, A.D., Cabodi, M., Bonassar, L.J., "A diffusively permeable monolithic biomaterial with embedded channels". Filed October 17, 2005.

2) Provisional: Stroock, A.D., Kirtland, J.D., Abruna, H.D., "Methods and Apparatus for symmetrically stirred laminar flow." Filed May 2006.

Awards:

2005 Invitation to "Frontiers of Engineering Symposium" of the National Academy of Engineering
2006 3M Non-Tenured Faculty Award
2006 Arnold and Mabel Beckman Young Investigator Award